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ORIGINAL ARTICLE

Synthesis and in-silico molecular docking simulation of 3-chloro-4-substituted-1-(2-(1*H*-benzimidazol-2-yl)phenyl))-azetidin-2-ones as novel analgesic anti-inflammatory agent

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Molecular docking

Abstract In the present investigation synthesis of some novel 1-(2-(1*H*-benzimidazol-2-yl)phenyl)-3-chloro-4-(Un/substitutedphenyl)azetidin-2-one (**3a**–**3h**) is reported. All these compounds were characterized by IR, Mass, ^{1}H NMR and elemental analysis. The newly synthesized compounds were screened for analgesic and anti-inflammatory activities on acetic acid induced writhing in mice and carrageenan induced paw edema in rats. Compound 3 g was found to have potent analgesic (46% at 20 mg/kg b.w) and anti-inflammatory (66.5% at 20 mg/kg b.w) activities as compared to standard drug nimesulide (20 mg/kg b.w). To check binding modes and binding affinity of synthesized compounds were docked into the active sites of enzyme COX-II. Compounds 3a, 3e and 3 h were found to have good affinity for COX-II. A good correlation is found between in silico docking analysis and in biological screening.

1. Introduction

Benzimidazoles and their analogs are well known biologically active *N*-containing heterocycles (Kozo et al., 2001), widely used as drugs such as proton pump inhibitor (Omeprazole) (Langtry and Wilde, 1998), antihelmentic (Albendazole) (Hazelton et al., 1995), and anti-dopaminergic Domperidone (Kennis et al., 1986) anti-psychotic (Pimozide) (Meisel et al., 1987). Specifically, the 2-substituted analogs of benzimidazoles are known to be potent biologically active compounds (Kazimierczuk et al., 2005) Apart from these benzimidazole nucleus is also found to be a part of analgesic and anti-inflammatory agents (Sondhi et al., 2002; Zheng et al., 2007; Sondhi et al., 2006; Kavitha et al., 2010; Charlson, 1975). Azetidinones have attracted much attention owing to their important pharmacological properties; a large numbers of azetidinones were reported to possess anti-inflammatory activity (Agarwal et al., 1989;

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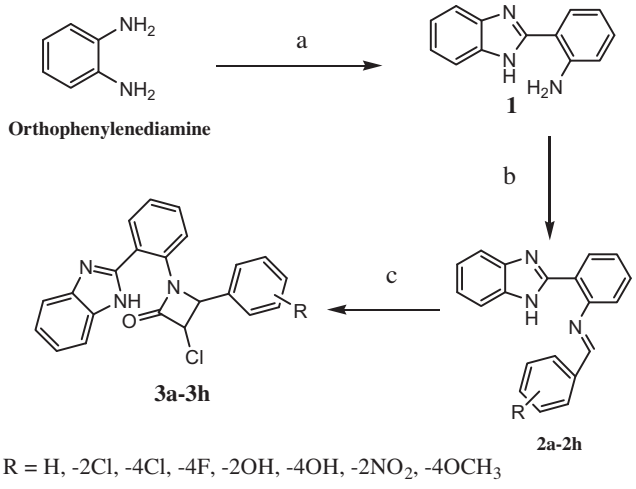
Srivastava et al., 2000). In the light of these observations this prompted us to synthesize a new series of benzimidazole derivatives by incorporating the azetidinone moiety at 2nd position of benzimidazole nucleus. In the present work, synthesis of some novel 1-(2-(1*H*-benzimidazol-2-yl)-phenyl)-3-chloro-4-[un/substituted phenyl]-azetidin-2-ones is reported. The structures of all compounds have been evaluated by elemental analysis and spectral analysis (IR and ^{1}H NMR). All the compounds have been screened for their analgesic and anti-inflammatory activity. In-silico molecular docking analysis of synthesized scaffold into the active site of enzyme cyclooxygenase is carried out.

2. Results and discussion

2.1. Chemistry

Target compounds **3a–3h** were prepared by synthetic protocol described in Scheme 1. 2-(1*H*-benzimidazol-2-yl)benzenamine (**2**) was synthesized by refluxing orthophenylenediamine with anthranilic acid in the presence of polyphosphoric acid. Obtained, compound **2** was then refluxed with different aromatic aldehydes in the presence of sulfuric acid to form Schiff bases (**2a**–**2h**). Resultant Schiff bases were cyclized to form 2-azetidinones **3a–3h** with chloro acetyl chloride in the presence of triethylamine in dry 1,4-dioxane. Triethylamine was used to absorb released hydrochloric acid. All reactions were monitored by analytical thin layer chromatography. The structures of synthesized compounds were confirmed by IR, MS and ^{1}H NMR spectroscopy. The IR spectra of compounds **2a–2h** provide valuable information regarding the nature of functional group present in the structure of compounds. The bands around 1595–1610 cm^{-1} suggested the presence of azomethine linkage in the compounds. The ^{1}H NMR spectra for compounds **2a–2h** showed a single peak for the azomethine ─CH═N proton, which varied in its position. The signals of ^{1}H NMR and IR were in complete agreement with the assigned structures to compounds **3a–3h**. From the IR spectra of compounds **3a–3h**, it can be concluded that the absence of stretching frequencies for azomethine linkage afforded the cyclization. The presence of >C═O peak around 1650–1750 cm^{-1} in the IR spectrum, confirms the achievement of cyclic amide ring in the compounds **3a–3h**. The mass spectra of these compounds displayed a molecular ion peak at appropriate m/z values which were corresponding well with the respective molecular formulas. All the compounds have given the satisfactory elemental analysis by C, H and N.



Scheme 1 Synthetic protocol for the target compounds **3a**–**3h**. Reagents: (a) anthranilic acid, polyphosphoric acid, reflux 4 h; (b) H_2SO_4, different aldehydes reflux; (c) 1,4 dioxane, chloroacetic acid, tryethylamine, reflux.

2.2. Pharmacology

2.2.1. Statistical analysis

Results of all the above estimations have been indicated in terms of mean ± SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett's test multiple comparisons test. The level of significance was set at $P < 0.05$.

2.2.2. Experimental animals

Albino rats of Wistar strain (150–200 g) and Swiss albino mice (25–30 g) of either sex were used in the entire study and were procured from Yash farm, Pvt. Ltd, Pune, India. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2 °C; relative humidity 60–70%) in a 12 h light–dark cycle. The animals were fed with standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during the experimental hours. The experimental protocol was approved by Institutional Animal Ethics Committee. All rats treated with different compounds up to 250 mg kg^{-1} were alive during the 24 h of observation. These animals did not show visible signs of acute toxicity. The tested compounds are considered non-toxic up to dose 250 mg/kg.

2.2.3. Analgesic activity

Pain reactivity was measured by the writhing test of Koster et al. Koster and Anderson (1959) The test was performed in mice by i.p. injection of a 0.6% acetic acid (1.0 ml/kg i.p.) 60 min after the administration of compounds. Each compound was administrated orally as a suspension of 1% Tween 80 of 20 mg kg^{-1}. Control groups received an equal volume of vehicle. Each experimental group consisted of five animals. Animals were placed in glass cages 5 min after acetic acid injection and number of writhes per animal was counted in the following 20 min. Nimesulide (20 mg/kg, p.o.) was used as a reference drug. Percentage protection against writhing was taken as an index of analgesia. Results are summarized in the Table 1.

2.2.4. Anti-inflammatory activity

Synthesized compounds **3a–3h** were tested for their in vivo anti-inflammatory efficacy using carrageenan-induced paw edema method (Winter et al., 1962). The tested compounds and the standard drug nimesulide produced significant reduction of paw size as compared to the control group. Rats in groups of five each were treated with vehicle, synthesized compounds **3a–3h** (20 mg/kg, p.o.) one hour prior to Carrageenan injection. 0.1 ml of 1% Carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swellings of carrageenan injected foot were measured at 0, 1, 2, 3 h using Plethysmometer (UGO Basile, Italy). The right hind paw was

Table 1 Analgesic activity of tested compounds (20 mg/kg b.w) and Nimesulide (20 mg/kg b.w).

Treatment	Number of Writhing	% Inhibition
Control	63.2 ± 1.020	–
Nimesulide	30 ± 1.183**	52.54
3a	39.6 ± 3.66**	37.34
3b	48.4 ± 1.94**	26.32
3c	50.6 ± 0.40**	19.93
3d	44.2 ± 1.114**	30.06
3e	34 ± 2.34**	46.20
3f	46 ± 1.897**	27.21
3g	49.2 ± 0.583**	22.15
3h	35.6 ± 0.509**	43.67

Data represent mean values SEM of five mice per group, shown at the final value for each group (control, nimesulide and tested compounds) after 1 h.
Data were analyzed using one-way ANOVA followed by Dunnett test.
** $p < 0.01$.

injected with 0.1 ml of vehicle. Nimesulide (20 mg/kg p.o.) was used as reference agent. Results are summarized in the Table 2.

2.3. Molecular modeling studies

The binding models of synthesized scaffold **3a–3h** in the enzyme active site of COX-II were depicted in Fig. 1. To pre-assess the anti-inflammatory behavior of our benzimidazole derivatives **3a–3h** on a structural basis automated docking studies were carried out using MDS v 3.5 program (Chhajed et al., 2010). The scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the COX-II enzyme (pdb code: 3NT1) (Kelsey and Duggan, 2010). Genetic algorithm implemented in MDS has been successfully employed to dock inhibitors into the catalytic site of the COX-II and to well correlate the obtained binding score with inhibitory activities of compounds. Obtained results were evaluated in terms of binding score into the catalytic site of COX-II. Smaller dock score indicates larger binding affinity of ligand for receptor. Results are summarized in the Table 3.

3. Conclusion

Compounds 3-chloro-4-substituted-1-(2-(1*H*-benzimidazol-2-yl)phenyl))-azetidin-2-ones **3a–3h** can be easily prepared in good yields. Purity of the compound was ascertained by analytical thin layer chromatography. Structures of synthesized compounds were confirmed by analytical (C, H and N) and spectral techniques (IR, MASS and ^{1}H NMR). Further compounds were screened for their analgesic and anti-inflammatory activity. Compound 1-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-3-chloro-4-(4-hydroxyphenyl)azetidin-2-one (**3e**) is found to be possess good analgesic and anti-inflammatory activity i.e.% analgesia is 46.02 as compared to 52.54 of nimesulide and 66.5% anti-inflammatory activity as compared to 51% of standard drug nimesulide. Compound 1-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-3-chloro-4-(4-methoxyphenyl)azetidin-2-one **3h** is found to possess good analgesic activity i.e. 43.67% and 46.7% anti-inflammatory activity. It is clear from the biological screening that para hydroxy and para methoxy substituted phenyl ring at fourth position of azetidinone ring is essential for analgesic and anti-inflammatory activity. Halogen substituted phenyl ring present at fourth position of azetidinones results in compounds with poor analgesic and anti-inflammatory activity. Further unsubstituted phenyl ring present at fourth position of azetidinones results in compound with moderate analgesic and anti-inflammatory activity.

Compounds **3a–3h** were docked into the active site of enzyme COX-II. Binding energy (dock score) obtained for compounds 3a, 3e, 3 h, and standard are −10.32, −11.82, −12.74 and −10.11, respectively against COX-II enzyme. Smaller dock score (binding energy) shows strong affinity toward the receptor. Compounds 3a, 3e, and 3 h have good affinity for receptor COX-II. It is evident from the pharmacological screening data that compounds 3e, and 3 h are potent analgesic anti-inflammatory agents where as compound 3a is a moderately active analgesic and anti-inflammatory agent as

Table 2 The anti-inflammatory activity of the tested compounds (20 mg/kg b.w) and Nimesulide (20 mg/kg b.w).

Treatment	Mean increase in paw volume (mL)				% Decrease in paw volume at 3 h
	0 h	1 h	2 h	3 h	
Control	0.61 ± 0.01	1.18 ± 0.009	1.37 ± 0.009	1.63 ± 0.01	–
Nimesulide	0.55 ± 0.007	0.61 ± 0.009**	0.74 ± 0.008**	0.81 ± 0.006**	51
3a	0.544 ± 0.01	0.58 ± 0.015**	0.73 ± 0.02**	0.94 ± 0.03**	43.4
3b	0.53 ± 0.013	0.73 ± 0.01*	0.88 ± 0.01**	1.28 ± 0.04**	21.5
3c	0.542 ± 0.012	0.65 ± 0.03**	0.97 ± 0.02**	1.26 ± 0.02**	22.7
3d	0.534 ± 0.012	0.58 ± 0.018**	0.91 ± 0.06**	1.27 ± 0.03**	22.02
3e	0.55 ± 0.015	0.57 ± 0.0012**	0.64 ± 0.012**	0.71 ± 0.01**	66.5
3f	0.48 ± 0.018	0.54 ± 0.015**	0.73 ± 0.021**	0.85 ± 0.01**	41
3g	0.562 ± 0.001	0.60 ± 0.02**	0.74 ± 0.01**	0.92 ± 0.01**	44.5
3h	0.55 ± 0.012	0.61 ± 0.006**	0.66 ± 0.02**	0.87 ± 0.03**	46.7

Data represent mean values SEM ± of five mice per group and the percent changes versus 1, 2 and 3 h post-carrageenan injection.
Data were analyzed using one-way ANOVA followed by Dunnett test **$p < 0.01$.
Percent edema inhibition was calculated as regards control group.
SEM-Standard Error of Mean.
The P value is < 0.0001, considered extremely significant.
* Significant difference from the control value at $p < 0.001$.
** Significant difference from the control value at $p < 0.01$.

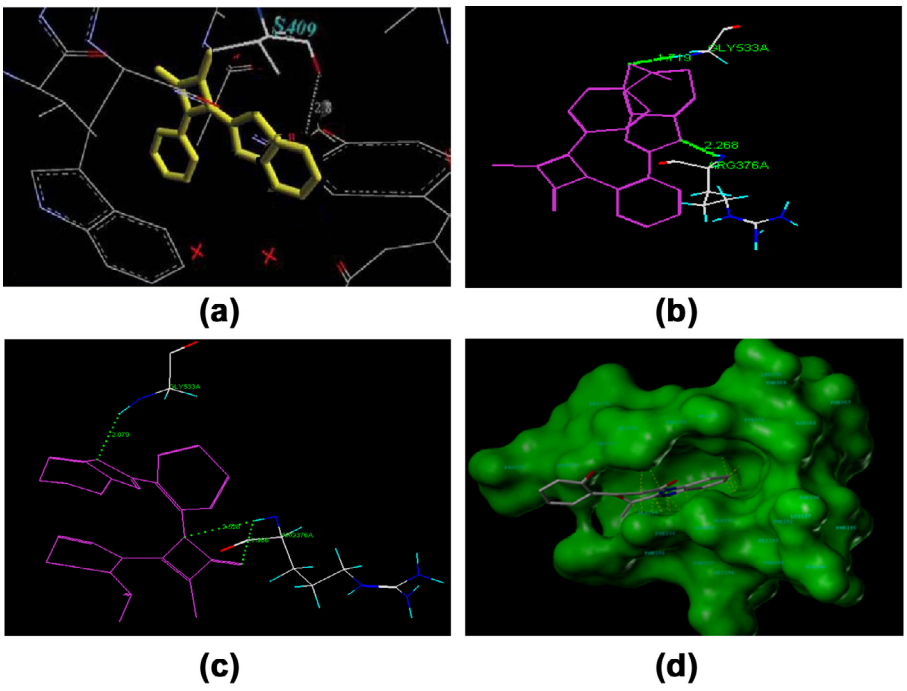


Figure 1 Best poses, orientations, hydrogen bond formed, and charge interactions of synthesized compounds 3a–3h with enzyme COX-II. (a) Molecule **3a** into the active site of enzyme COXII. It has binding score of −10.32 and shows hydrogen bonding between O of 2-oxo of 2-azetidinone ring and 409 Serine. (b) Molecule **3h** into the active site of enzyme COX-II showing hydrogen bonding between O of methoxy and N of benzimidazole ring with glycine 533 and arginine 376, respectively. It has binding score −12.74 kJ/mole. (c) Molecule **3f** into the active site of enzyme showing hydrogen bonding between nitrogen and oxygen of azetidinone with arginine 376 and also between nitrogen of benzimidazole ring and glycine 533. (d) Molecule **3b** into the active site of enzyme CoX-II.

Table 3 Molecular docking showing binding score of synthesized compounds in the active site of enzymes COX-II.

Treatment	Dock score*
Nimesulide	−10.11
3a	−10.32
3b	−06.10
3c	−09.37
3d	−04.83
3e	−11.82
3f	−07.01
3g	−07.18
3h	−12.74

* Dock score is binding energy in kilojoules per mole.

compared to standard nimesulide. In conclusion good correlation is found between in silico molecular docking experiments and biological screening.

4. Experimental

4.1. Materials and methods

Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a Jasco infrared spectrometer in KBr. ^{1}H NMR was recorded by a Brucker Avance II 400 spectrophotometer at a frequency of 400.13 MHz. The electrospray mass spectra were recorded on MICROMASS QUATRO II triple quadrapole mass spectrophotometer. All the synthesized compounds were analyzed satisfactorily for C, H and N by Elementar Vario EL III elemental analyzer. Analytical thin layer chromatography (TLC) was performed by using adsorbent silica gel G, visualization of the developed chromatogram was performed with iodine vapors. Solvents and reagents obtained from commercial sources were used without purification.

4.2. Synthesis of 2-(1H-benzimidazol-2-yl)-benzenamine (1)

In 250 ml round bottom flask, 0.01 M (1.08 g) O-phenylenediamine was taken; to it was added 20 mL of ethylene glycol. O.03 M (4.11 g) anthranilic acid and 2–3 drops of polyphosphoric acid ware added. The reaction mixture was heated on sand bath at about 190–195 °C for 4 h. The reaction mixture was poured into crushed ice. Precipitate formed was filtered, washed with cold water, dried and recrystallized from ethanol. Yield: (77%). M.p: 190–192 °C, R_f : −0.62 [benzene: ethyl acetate (9:1)], IR (KBr, cm^{-1}) 3360.41–3270.26 (asymmetric and symmetric N—H str respectively), 1609.02 (N—H def),1250.33 (C—N str). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.9 (s, 1*H*, —NH—), δ 6.6–8.9 (M, 8 Ar—H) δ 3.97 (s 2H, Ar—NH_2).

4.3. General procedure for synthesis of 2-(1H-benzimidazol-2-yl)-N-[un/substituted-benzylidene] benzenamine

Equimolar quantities of unsubstituted/substituted aromatic aldehydes and 2-(1*H*-benzimidazol-2-yl)-benzenamine were dissolved in 20 mL of warm dry ethanol. To it was added 1–2 drops of concentrated sulfuric acid and heated at reflux

for 3 h on water bath. After standing for approximately one hour at room temperature (r.t), the crystalline product was separated by filtration, dried.

4.3.1. 2-(1H-benzimidazol-2-yl)-N-benzylidenebenzenamine (2a)

$C_{20}H_{15}N_3$, Yield 76%, m.p: 237–239 °C, IR (KBr cm^{-1}) 3260.38 (N—H str ring), 3030.21 (C—H str aromatic), 1635.27 (C=N str imine), ^{1}H NMR: δ 6.6–9.1 (m, Ar—H), δ 8.11 (s, 1*H*, N=CH—), δ 5.01 (s, ring N—H). **m/e:** 297.

4.3.2. N-(2-chlorobenzylidene)-2-(1H-benzimidazol-2-yl)benzenamine (2b)

$C_{20}H_{14}ClN_3$, Yield 65%, m.p: 154–156 °C, IR (KBr cm^{-1})**:** 3310.35 (N—H str ring), 3134.37 (Aromatic C—H str), 1653.07 (C=N str of imines), 750.23 (C—Cl str). **^{1}H NMR:** δ 6.5–8.7 (m, 12H, Ar—H), δ 8.12 (s, N=CH—), δ 5.08 (s, —N—H). **m/e:** 331.

4.3.3. N-(4-chlorobenzylidene)-2-(1H-benzimidazol-2-yl)benzenamine (2c)

$C_{20}H_{14}ClN_3$, Yield 57%, m.p: 202–204 °C, IR (KBr cm^{-1}): 3066.03 (aromatic C–H stretching) 1648.62 (C=N str of imines), 1657.35 (aromatic C=C str), 645.54 (C—Cl str). ^{1}H NMR: δ 6.8–8.8 (m, 12H, Ar—H), δ 7.16 (s, N=CH—), δ 4.02 (s, 1*H*, —N—H), **m/e:** 331.

4.3.4. N-(4-fluorobenzylidene)-2-(1H-benzimidazol-2-yl)benzenamine (2d)

$C_{20}H_{14}FN_3$Yield 66%, m.p: 176–178 °C, IR (KBr cm^{-1}): 3317.26 N—H str (Sec Amines), 1642.89(Aromaic C=C str), 1500.33 N—H def. 1656.76 C=N str imine), ^{1}H NMR: δ 8.0 (s, N=CH—), δ 6.5–7.5 (m, 12H, Ar—H), δ 5.1 (s, —N—H). **m/e** = 316.

4.3.5. 4-[-{[2-(1H-benzimidazol-2-yl)phenyl]imino}methyl] phenol(2e)

$C_{20}H_{15}N_3O$, Yield 70%, m.p: 259–261 °C, IR (KBr cm^{-1}) 3509.42 (O—H stretching), 3460.92 (N—H stretching ring), 3030.31 (Aromatic C–H stretching), 1690.62 (Imines C=N Stretching), ^{1}H NMR: δ 9.86 (s, 1*H*, Ar—OH), δ 7.13 (s, N=CH—), δ 6.5–7.7 (m, 12H,Ar—H), δ 3.92 (s, 1*H*, ring-NH), **m/e:**313.

4.3.6. 2-[-{[2-(1H-benzimidazol-2-yl)phenyl]imino}methyl] phenol (2f)

$C_{20}H_{15}N_3O$,Yield 70%, m.p: 122–124 °C, IR (KBr cm^{-1})= 3398.67 O—H str. 3252.22 N—H str (Sec Amines), 1651.10 (Aromaic C=C str), 1664.00 C=N str imine) 1500.33 N—H def. (sec amines), 1298.83 C—N str.

^{1}H NMR: δ 9.52 (s, 1*H*, Ar—OH), δ 6.5–9.2 (m, 12H,Ar—H), δ 8.12 (s, N=CH—), δ 5.02 (s, 1*H*,Ar—O—H), δ 4.00 (s, 1*H*,ring N—H), **m/e:**313.

4.3.7. N-(2-nitrobenzylidene)-2-(1H-benzimidazol-2-yl) benzenamine(2g)

$C_{20}H_{14}N_4O_2$,Yield 70%, m.p: 77–79 °C, IR (cm^{-1}): 3460.84 (N—H str ring), 3030.21 (C—H str aromatic), 1690.27–1590.83 (C=N str imine), 1555.91–1487.11 (N=O str), ^{1}H NMR: δ 6.5–8.5 (m, 12H, Ar—H), δ 7.00 (s, N=CH—), δ 5.02 (s, 1*H*, —N—H), **m/e:** 342.

4.3.8. N-(4-methoxybenzylidene)-2-(1H-benzimidazol-2-yl)benzenamine (2h)

$C_{21}H_{17}N_3O$, Yield 65%, m.p: 240–242 °C, **IR** (KBr cm^{-1}): 3509.22 (N—H stretching) 3029.03 (Aromatic C–H stretching), 1693.23 (imines C=N stretching), 1460.27 (—OCH_3 stretching), 1100.62 (C—O str). **^{1}H NMR:** δ 6.8–9.1 (m, 12H, Ar—H), δ 8.85 (s, N=CH—), δ 3.8 (s, 3H,—OCH_3), δ 3.8 (s, 1*H*,—N—H). **m/e:** 328.

4.4. General procedure for synthesis of 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-[(un)/substituted phenyl)]azetidin-2-one

To 0.01 M various 2-(1*H*-benzimidazol-2-yl)-*N*-[Un/substitutedbenzylidene]benzenamines, was added 20 ml of 1,4 dioxane.The mixture was warmed to dissolve, the resultant solution was allowed to cool, and to it was added 0.01 M triethyl amine and 0.01 M chloroacetyl chloride dropwise and with stirring. Mixture was refluxed on the boiling water bath for 4 h. Allowed to cool and filtered at pump, air dried.

4.4.1. 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-phenylazetidin-2-one (3a)

$C_{22}H_{16}ClN_3O$, Yield: 76%, m.p: 276–278 °C, IR (KBr cm^{-1}): 3061.06 (aromatic C—H str), 1751.42(C=O str amides),1248.67 600 (C—lCl str). ^{1}H NMR ($CDCl_3$): δ 6.9–8.3 (m, 13H, Ar—H) δ 5.7 (s, 1*H*, C3—H), δ 5.4 (s, 1*H*, C4—H), δ 4.4 (s, 1*H*, —NH), **m/e:** 373, C=70.61%, H=, 4.30%, and N = 11.19%.

4.4.2. 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-(2-chlorophenyl)azetidin-2-one (3b)

$C_{22}H_{15}Cl_2N_3O$, Yield: 65%, m.p: 154–156 °C, IR (KBr cm^{-1}): 3232.80 (N—H stretching secondary amine), 3066.06 (Aromatic C—H str), 1749.49 (C=O str amides), 734.90 (C—Cl str). ^{1}H NMR ($CDCl_3$): δ 6.7–8.6(m, 12H,Ar—H), δ 5.5 (s, 1*H*, C3—H), δ 5.2 (s, 1*H*, C4—H), δ 4.2 (s,1*H*,—N—H), **m/e:**408, C = 64.69%, H=, 3.66%, and% N = 10.25.

4.4.3. 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-(4-chlorophenyl)azetidin-2-one (3c)

$C_{22}H_{15}Cl_2N_3O$, Yield: 57%, m.p: 202–204 °C, **IR** (cm $^{-1}$) = 3314.66(N—H str), 1747.34(C=O str of cyclic amide), 3119.28 (aromatic C—H str) 2934.72 and 2889.02(asymmetric and symmetric C—H str), 786.34 (C—Cl str). ^{1}H NMR ($CDCl_3$): δ 6.7–8.6(m, 12H, Ar—H) δ 5.2 (s, 1*H*, C3—H), δ 4.9 (s, 1*H*, C4—H), δ 4.1 (s, 1*H*, N—H), **m/e:** 408, C = 64.67%, H=, 3.69%, and N = 10.17%.

4.4.4. 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-(4-fluorophenyl)azetidin-2-one (3d)

$C_{22}H_{15}ClFN_3O$, Yield: 60%, m.p: 109–111 °C, IR (KBr cm^{-1}): 3353.98 (N—H str), 3151.47 (Aromatic C—H str), 2956, 3008.35 (asym. and sym. C—H str), 1733.48 (C=O str amides).

^{1}H NMR ($CDCl_3$): δ6.8–8.5(m, 12H, Ar—H), δ 5.58 (s, 1*H*, C3—H), δ 5.09 (s, 1*H*, C4—H), δ 4.10 (s, 1*H*, N—H), **m/e:** 391, C = 67.37%, H=, 3.85%, and N = 10.69%.

4.4.5. 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-(2-hydroxyphenyl)azetidin-2-one (3e)

$C_{22}H_{16}ClN_3O_2$, Yield: 70%, m.p: 92–94 °C, IR (KBr cm^{-1}): 3305.12 (O—H str), 3157.36 (Aromatic C—H str), 2937.82

and 2900.28 (asym.and sym. C—H str), 1749.32 (C = 0 str of amides), 600 (C—Cl str) ^{1}H NMR ($CDCl_3$): δ 9.62 (s, 1*H*, Ar—OH), δ 6.7–7.9 (m, 12H, Ar—H), δ 5.44 (s, 1*H*, C3—H), δ 5.00 (s, 1*H*, C4—H), δ 4.20 (s, 1*H*, —NH), **m/e:**389, C = 67.77%, H =, 4.39%, and N = 10.74%.

4.4.6. 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-(4-hydroxyphenyl)azetidin-2-one (3f)

$C_{22}H_{16}ClN_3O_2$, Yield: 55%, m.p: 197–199 °C, IR (KBr cm $^{-1}$):3314.66 (O—H str), 1747.34 (C=O str of cyclic amide), 3119.28 (Aromatic C—H str) 2934.72 and 2889.02 (asymmetric and symmetric C—H str), 786.34 (C—Cl str). ^{1}H NMR ($CDCl_3$): δ 9.99 (s, 1*H*, Ar—OH), δ 6.7–7.9 (m, 12H, Ar—H), δ 5.44 (s, 1*H*, C3—H), δ 5.00 (s, 1*H*, C4—H), δ 4.10 (s, 1*H*, —NH), **m/e:** 389, C = 67.75%, H =, 4.37%, and N = 10.69%.

4.4.7. 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-(2-nitrophenyl)azetidin-2-one (3g)

$C_{22}H_{15}ClN_4O_3$, Yield: 70%, m.p: 123–125 °C, IR (KBr cm^{-1}): 3377.45 (N—H str), 3099.7 (aromatic C—H str), 2951.19 and 2912.61 (asym.and sym. C—H str), 1739.13 (C = 0 str of amides), 638.46 (C—Cl str),1508.38 and 1473.66 (N=O str asymmetric and symmetric), ^{1}H NMR ($CDCl_3$)**:** δ 6.9–8.4 (m, 12H, Ar—H), δ 5.8 (s, 1*H*, C3—H), δ 5.6 (s, 1*H*, C4—H), δ 3.85 (s, 1*H*, —NH), **m/e:**418, C = 63.05%, H =, 3.59%, and N = 13.35%.

4.4.8. 1-(2-(1H-benzimidazol-2-yl)phenyl)-3-chloro-4-(4-methoxyphenyl)azetidin-2-one (3h)

$C_{23}H_{18}ClN_3O_2$, Yield: 45%, m.p: 67–69 °C, IR (KBr cm^{-1}): 3260.35 (N—H stretching ring), 3151.79 (aromatic C—H str), 1695.07 (C=O str of amide), 1255 and 1045.45 (C—O str of phenyl alkyl ether). 750.42 (C—Cl Str) ^{1}H NMR ($CDCl_3$): δ6.9–8.9 δ 5.5 (s, 1*H*, C3—H), δ 5.1 (s, 1*H*, C4—H), δ 4.41 (s, 1*H*, —NH), (m, 12H, Ar—H), δ 3.74 (s, 3H, —OCH_3), **m/e:** 403, C = 68.33%, H =, 4.46%, and N = 13.37%.

4.5. Pharmacology

4.5.1. Analgesic activity

The analgesic validation of tested compounds was evaluated using the acetic acid-induced writhing method. Mice (25–30 g) were used in groups, each containing five animals. The 1st group was kept as a control (received the vehicle) while the 2nd one was subcutaneously injected with nimesulide in a dose of 20 mg/kg (standard). The other groups were injected with the tested compounds in doses 20 mg/kg body weight. Writhing was induced 30 min later, by intraperitoneal injection of 0.1 ml of 0.6% acetic acid. Numbers of writhes (abdominal contractions) in all animals were counted for 20 min, immediately after acetic acid injection (0 time) and hourly after administration for 4 h. Analgesic activity was expressed as the percentage protection against writhing produced by the new compounds all over the experimental period comparing between control and those pretreated with the new compounds using the ratio,

[(No of writhing in control group – No of writhing in treated group)/No of writhing in control group]×100.

4.5.2. Anti inflammatory activity

The anti-inflammatory effect of the newly synthesized compounds was evaluated in correspondence to the carrageenan-induced paw edema method. Groups of animals each consisting of five rats weighing 180–200 g were used. The 1st group was treated with the vehicle and left as control while the 2nd one was given nimesulide by subcutaneous injection in a dose of 20 mg/kg body weight. Other groups were administered orally the tested compounds in doses 20 mg/kg body weight. After 30 min, acute inflammation was induced by subplantar injection of 0.1 ml of 1% suspension of carrageenan in the right hind paw of all rats. The volume of the paw was measured plethysmographically immediately 1, 2 and 3 h after the injection of the irritant. The difference in volume gave the amount of edema developed. Percent inhibition of the edema between the control group and compound treated groups was calculated and compared with that of the group receiving a standard drug.

4.6. In-silico molecular docking

Molecular docking of compounds **3a–3h** into the three-dimensional X-ray structure of COX-II (PDB code: 3NT1) was carried out using the molecular design Suite (MDS) software package (v. 3.5). The protein–ligand complex was constructed based on the X-ray structure COX-II. All compounds were built using Chem Draw Ultra v.8.0 and minimized using the Merck Molecular Force Field. Keeping program parameters to their default values, the docking was performed using Molecular Design Suite (MDS) into the 3D model of the catalytic site of COX-II. Genetic algorithm implemented in MDS has been successfully employed to dock inhibitors into the catalytic site of the enzyme. The obtained binding score was found to be in good agreement with inhibitory activities of respective compounds. Comparative docking experiments of compounds **3a–3h** with known COX-II inhibitor agent such as nimesulide were performed. Obtained results were evaluated in terms of binding score into the catalytic site of enzymes.

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